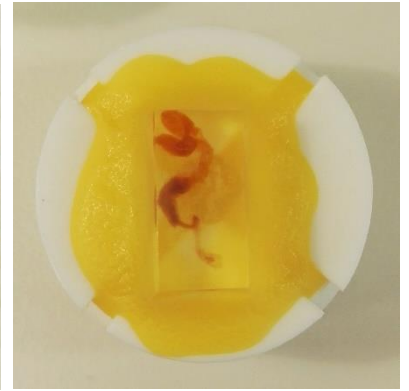


Morphology and localization of carbonic anhydrase in the alimentary canal of the Swedish house cricket, *Acheta domesticus* (Orthoptera: Gryllidae)

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Morphology and localization of carbonic anhydrase in the alimentary canal of the Swedish house cricket, *Acheta domesticus* (Orthoptera: Gryllidae)

Digestionskanalens morfologi och lokalisering av karbanhydras hos den svenska hussyrsan, *Acheta domesticus* (Orthoptera: Gryllidae)

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SUMMARY

There is a growing interest in using insects as part of the human diet. Actors such as the Swedish University of Agricultural Sciences (SLU) and Nordic Food Labs are investigating the potential use of insects as a source of protein and nutrients for the western human population. This degree project in veterinary medicine is part of the SLU project; *Eating crickets- an appetizing solution for today's global problems* which aims to develop a new sustainable protein source based on the Swedish house cricket, *A. domesticus*. Important aspects within the project are sustainability, biodiversity, nutrition, ethics, food safety and acceptance (SLU 2017). To produce a food that achieves this, we need to know more about the food source itself. How does the metabolism of the house cricket work and more precisely how does its alimentary canal work? This project aims to describe the morphology of the alimentary canal of the Swedish house cricket and to investigate the activity of the enzyme family of carbonic anhydrases (CA) along its entire length.

In the main study 20 conventionally bred crickets, at the age of 40-45 days, were used. The crickets were divided into two groups, equal distribution in terms of sex, and one group was left to starve for 36h whereas the other group was fed. Apart from this treatment the same protocol was used for both groups. After being euthanized (anesthetized with CO₂ and then decapitated) the alimentary canal was dissected, anatomical data was collected, and the tissue samples were prepared for histological examination. Segments from all parts of the gut were stained with HE and for carbonic anhydrase. Slides were evaluated using light microscopy.

In this study the alimentary canal of *A. domesticus* has for the first time been described in its entirety, both anatomically and histologically. The alimentary canal of *A. domesticus* is very similar to the gut of other species within the Gryllidae family. The results suggest the presence of goblet cells forming gastric glands in the midgut epithelium. To confirm the presence of goblet cells and investigate the constitution of the possible gastric glands additional histological examination with PAS-staining is needed. No significant results were obtained regarding differences between groups including males compared to females and fed individuals compared to starved. If the study is repeated more care should be taken to have crickets of as similar age as possible and increase the size of the groups. The localization of active CA has been demonstrated in the striated muscle along the entire alimentary canal and in the epithelium of the ceaca and ventriculus. CA activity is found in the membrane of the majority of the columnar epithelial cells in the midgut suggesting that CA is associated to the most numerous cell type, the enterocyte. In order to determine which cell type/ cell types that contains CA additional histological examination is needed. There are possible sex differences regarding CA activity in the midgut. Starved males showed none to weak black staining compared to the much stronger staining in the epithelium of starved females. To confirm the results of possible sex differences the study needs to be repeated with increased group sizes. It is suggested that CA in the alimentary canal of *A. domesticus* may contribute to acid-base balance in both muscle and gut lumen. Future experiments with ion selective microelectrodes and presence/ absence of CA inhibitors may be a way to test this hypothesis.

SAMMANFATTNING

Intresset för att använda sig av insekter som livsmedel till människor ökar. Insekter har sedan länge varit en naturlig del av människors diet i Afrika och Asien, men nu ökar intresset även i västvärlden. Aktörer så som Sveriges lantbruksuniversitet (SLU) och Nordic Food Labs jobbar med att utreda det potentiella användningsområdet för insekter som en källa till protein och näring för människan. Detta examensarbete är en del av SLU:s projekt "Äta insekter - en smaklig lösning på dagens globala problem" som handlar om att utveckla ett nytt svenskt, hållbart protein- och mineralrikt livsmedel baserat på svensk hussysa. Viktiga aspekter inom projektet är hållbarhet, biologisk mångfald, näringsinnehåll, etik, livsmedelssäkerhet samt acceptans (SLU 2017). För att kunna åstadkomma ett livsmedel som uppfyller detta behöver vi veta mer om hussysan. Hur tillgodogör den sig näring och hur fungerar dess mag-tarmkanal? Syftet med detta arbete är att dokumentera mag-tarmkanalens morfologi samt undersöka förekomsten av enzymp familjen karbanhydras i densamma hos den svenska hussysan.

Till huvudstudien användes 20st konventionellt uppfödda syrsor, 40 - 45 dagar gamla. Syrsorna delades in i två grupper, med jämn könsfördelning, varvid den ena gruppen lämnades utan foder i 36 timmar före avlivning. Båda grupperna behandlades därefter enligt samma procedur. Efter att ha avlivats, fridissekerades mag-tarmkanalen, anatomiska data samlades in och vävnaden preparerades inför histologisk undersökning. Segment från samtliga delar av mag-tarmkanalen färgades med HE och för karbanhydras och utvärderades med ljusmikroskop.

Mag-tarmkanalen hos den svenska hussysan har i denna studie beskrivits i sin helhet. Den svenska hussysans mag-tarmkanal liknar mag-tarmkanalen hos övriga syrsarter inom familjen Gryllidae. Resultatet tyder på förekomst av körtlar, bestående av bägarceller, i tarmkanalens mellersta avsnitt. För att bekräfta detta krävs ytterligare histologiska undersökningar med PAS-färgning. Inga signifikanta skillnader mellan de två grupperna eller kön kunde påvisas. Vid upprepning av studien bör större hänsyn tas till att använda syrsor i samma ålder samt att använda större grupper. Förekomst av aktivt karbanhydras kunde påvisas i muskulaturen som omger matstrupe, kräva, muskelmage, blindtarm, magsäck, tunntarm, ändtarm och anal. Karbanhydrasaktivitet förekommer även i blindtarmarnas och magsäckens epitellager. Membranbunden karbanhydrasaktivitet sågs hos majoriteten av blindtarmarnas och magsäckens epitelceller vilket tyder på att karbanhydras är associerad till den mest talrika celltypen, enterocyten. För att bestämma vilken celltyp/celltyper som innehåller karbanhydras behövs ytterligare histologiska undersökningar utföras. Det finns möjliga könsskillnader när det gäller karbanhydrasaktivitet i blindtarm och magsäck hos individer som svultit. För att bekräfta resultaten krävs större experiment med fler individer. Det föreslås att karbanhydras i hussysans mag-tarmkanal bidrar till syra/bas-balans i både muskler och tarmlumen. Framtida experiment med jonselektiva mikroelektroder och närvaro/frånvaro av karbanhydras-hämmare kan vara ett sätt att testa denna hypotes.

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INTRODUCTION

This degree project in veterinary medicine is part of the SLU project; *Eating crickets- an appetizing solution for today's global problems* which aims to develop a new sustainable protein source based on the Swedish house cricket, *Acheta domesticus*.

In most parts of the world insects are a natural component of the human diet, Europe and parts of North America being two exceptions (FAO 2013). Crickets are generalists and can thrive on various organic material (Makkar et al., 2014), converting, to humans, uneatable plants into valuable protein. Crickets are nutritious, they have both high trace mineral content (Rumpold & Schuter, 2013) and higher iron content than conventional red meat (FAO 2013). Crickets have a higher percentage of edible weight than both cattle and chicken (Nagaki & DeFoliat, 1991). The cricket can therefore potentially replace meat and fish and contribute to reduce the lack of protein and trace elements in humans in developing countries.

In the western world insects are often associated with disgust. This is a serious obstacle to override to achieve insect consumption in western societies (Rozin et al., 2014). However, western media and restaurants are already promoting insects as a new source of protein. In the Nordic parts of the world Nordic food lab is a big actor. Working with famous restaurant Noma (Hermansen 2012), in Denmark, and making an educational documentary series “Världens godaste insekter” shown by Utbildningsradion (2016) in Sweden are just two examples of projects they are running.

Knowledge about the house cricket is limited. In the literature solely the midgut of *A. domesticus* has previously been histologically documented (Ulrich 1981). As the western world's interest of insects as a new protein source is increasing new research is motivated. In order to be able to ethically create a safe, sustainable and nutritional protein source we need to know more about the species, including the morphology and physiology of the insect's alimentary canal.

Aim

This degree project in veterinary medicine aims to describe the morphology of the alimentary canal of the Swedish house cricket and to investigate the localization of the enzyme family of carbonic anhydrases along its entire length.

LITERATURE REVIEW

Insects

Arthropoda is the largest and most diverse animal phylum, including insects and three other major classes (chelicerates, myriapods and crustaceans). The primitive body of insects is segmented and divided into three main body regions; head, thorax and abdomen. All insects have three pair of legs and an exoskeleton and most of them have wings and antennae (Kendall 2014). Insects have an open circulatory system, haemolymph (plasma fluid and haemocytes) flows freely surrounding the insect's organs. Most insects have a major pump termed the dorsal vessel, representing the heart and aorta, pumping haemolymph through the body. Haemolymph both transports and stores hormones and nutrients. It is also a vital component of the insect immune system (Chapman 2013). The fat body, comparable to the vertebrate liver, is an organ exclusive to insects. The organ is disseminated throughout the insect body but concentrated to the gut and reproductive tissues. Insects breathe through spiracles (small openings in the body wall). Air filled tubes (tracheols) originating from the spiracles are transporting oxygen directly to the cells of the body (Klowden 2013).

The house cricket, A. domesticus

Within the class of insects, is the orthopteran order consisting of grasshoppers, crickets and katydids. The house cricket, *Acheta domesticus*, is a part of the Gryllidae family (true crickets) and belongs to the subfamily Gryllinae (field cricket) (Kendall 2014). Crickets are a close relative to grasshoppers. There are a few features to tell them apart. For instance, on the head of grasshoppers you can easily separate the front from the vertex, see Fig. 1. In crickets there is no clear border between the two (Strid et al., 2017). Crickets also have longer antennae and fewer tarsal segments than the grasshopper (Walker 2017). Male crickets sing by rubbing their forewings together, creating oscillations. The main purpose of male cricket singing is courting females and male-male interactions (Alexander 1962). The cricket chirps are higher pitched than those of the grasshopper and resemble birdsong (Strid et al., 2017). The cricket ear is located on each foretibia, the tibia of the first pair of legs, see Fig. 1 (Bailey 1993).

The house cricket originates from North America and the Middle East. The specie is present outdoors in southern parts of Sweden, and can be found indoors in practically any part of the country (Strid et al., 2017). The adult house cricket is 14-20 mm long (Strid et al., 2017) and have two sets of wings. The cricket's head is beige with three dark bands, the anterior one (between the antennae) being horse shoe shaped. The pronotum (neckpiece) see Fig. 1, as well as the wings are beige with brown and black markings. Legs are brown, and the abdomen is pale/beige (McLeod 2016). Crickets have three life stages; egg, nymph and adult. The female house cricket lays her eggs which have to mature before a nymph is hatched. The nymph is a tiny and simplified version of the adult cricket. In order to grow, the young cricket has to shed its exoskeleton, this process is called molting (Alexander 1968). Molting occurs up to 14 times before the cricket has reached adult size (Bate 1971). The development from egg to adult cricket takes about 50 days (Ismail 1978). The adult cricket has two sensory antenniform cerci at the rear end. Between these the female cricket has a third antenniform process, the ovipositor (Fox 2007), see Fig. 1.

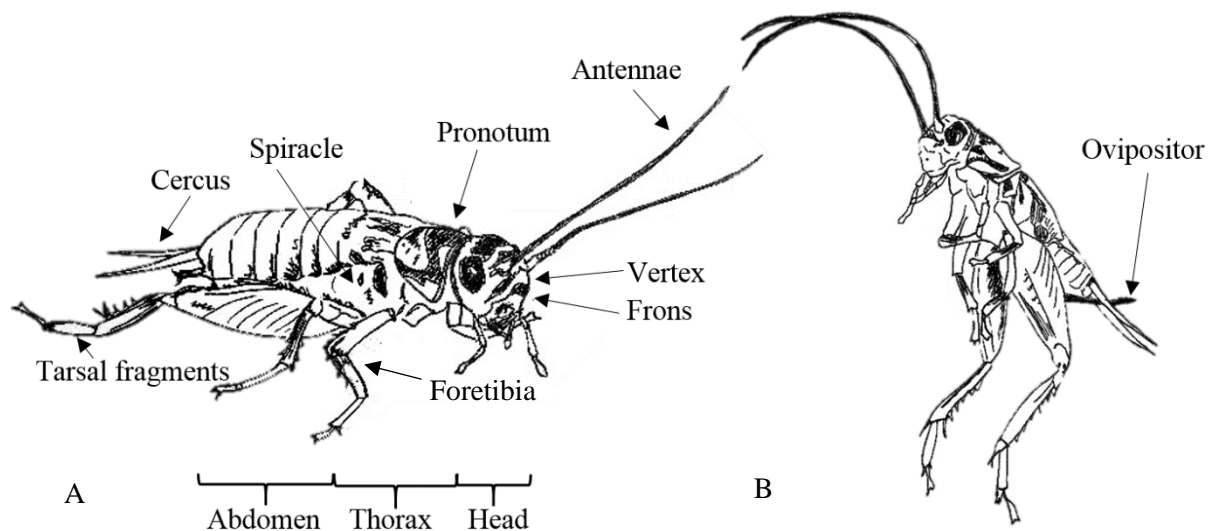


Fig. 1. Sketch of house crickets, *Acheta domesticus* (by author). A. Male nymph, age 30-35 days, approximately two molts before adult. B. Female nymph, age 30-35 days, approximately two molts before adult.

The alimentary canal of insects

Anatomy

The alimentary canal is a modified tube reaching from the mouth, through the entire length of the insect, to the anus. It is divided into three main parts, foregut, midgut and hindgut where the foregut and hindgut are of ectodermal origin whereas the midgut is of endodermal origin. The alimentary canal's main purpose is by processing ingested food provide the insect with essential energy, water and nutrients (Chapman 2013; Klowden 2013). There is a great diversity of insect feeding and the anatomical structures associated with feeding. The "typical" alimentary canal of insects does therefore not exist. Cockroaches, who are ancestral scavengers, have a relatively primitive gut and are often used as a prototype. The alimentary canal of cockroaches is divided evenly between storage, digestion and osmoregulation (Klowden 2013).

Foregut; pharynx, esophagus, crop and proventriculus.

The pharynx and esophagus are simple tubes leading to the crop. The crop is a storage organ that due to both longitudinal and transverse folds can stretch out and increase in size. The posterior end of the foregut, the proventriculus also called the gizzard, is variable in form, ranging from a simple sphincter to a well-developed gizzard with grinding function and regulating passage of food to the midgut (Chapman 2013). The salivary glands are located bilaterally in the thoracic region (Klowden 2013).

Midgut; ventriculus with adjacent caeca.

At the anterior end of the midgut there are a number of caecae, the rest of the midgut is called ventriculus (Chapman 2013). In most insects the malpighian tubules, a secretory organ of endodermal origin, located in the hemocoel, fuse with the alimentary canal at the junction between the midgut and the hindgut (Hazelton et al. 1988; Chapman 2013).

Hindgut; pylorus, ileum, colon, rectum and anus.

The midgut and the hindgut are separated by a sphincter (pylorus). In some species the posterior part of ileum can be differentiated from the anterior and is then termed colon. (Chapman 2013). Rectum, posterior of ileum, is wide and is where fecal pellets are formed (Chapman 2013; Klowden 2013).

Histology

The alimentary canal is composed of a single layer of epithelial cells supported by a basement membrane surrounded by striated muscle (Chapman 2013).

Foregut; pharynx, esophagus, crop and proventriculus.

The epithelial cells of the foregut are flattened and undifferentiated. The entire foregut is outlined by a cuticular lining, the intima, which may be sclerotized and covered with chitin teeth (Chapman 2013). The salivary glands are either tubular (in dipterans) or acinar (in cockroaches). The acini consist of three different cell types, peripheral cells, central cells and centroacinar cells (Klowden 2013).

Midgut; ventriculus with adjacent caeca.

The midgut epithelium consists of at least four different cell types and is not outlined by intima. The enterocyte (also called principle cell or columnar cell) is the most dominant cell. Scattered throughout the epithelium are also enteroendocrine cells and goblet cells. Groups of intestinal stem cells are organized in structures called nidi close to the basal lamina (Chapman 2013; Klowden 2013).

Hindgut; pylorus, ileum, colon, rectum, anus.

The hindgut epithelium, as the foregut epithelium, consists of flattened undifferentiated cells and is lined by sclerotized intima. In the region of the ileum, the cells show excessive apical folding (Chapman 2013; Klowden 2013). Rectum may have several rectal pads, being areas of the epithelium with columnar cells with locally thinner intima. These areas absorb water and ions leaving fecal pellets (Chapman 2013; Klowden 2013).

The peritrophic membrane

In most insects both cecae and ventriculus are lined with a noncellular membrane, the peritrophic membrane, also called the peritrophic envelope. The peritrophic membrane consists of a proteoglycan gel on a framework of chitin microfibrills. Included in the gel are peritrophins, a molecule similar to the mucins found in the alimentary canal of vertebrates. The membrane is permeable to inorganic ions and small organic molecules. The main purpose of the peritrophic membrane is to act as a protective barrier towards damage, pathogens and toxins as well as contribute to the transport of luminal content, as it sheds it moves towards the hindgut (Chapman 2013). The endoperitrophic space (between the ventricular epithelium and the peritrophic membrane) and the ectoperitrophic space (in the gut lumen) works as compartments for digestive enzymes, increasing digestibility (Chapman 2013; Klowden 2013).

Muscles of the alimentary canal and transport of gut content

Muscles are present along the entire alimentary canal and arise on the body wall (extrinsic visceral muscles) or are associated to the gut epithelium (intrinsic visceral muscles). Both types of muscles are striated and resemble typical skeletal muscles. Extrinsic visceral muscles are found in the foregut and hindgut and function as dilators. Intrinsic visceral muscles are well developed in the foregut and poorly developed in the midgut. The intrinsic muscles are arranged in one inner longitudinal and one outer circular layer. Contraction of the circular muscle layer results in longitudinal folds of the gut epithelium. Together with contraction of the longitudinal muscles peristalsis occur. The muscle groups have antagonistic functions which results in a pumping movement moving gut content along the gut towards anus. The transport of midgut content is aided by the peritrophic membrane. As new laminae are continuously produced old ones move back with food resulting in more laminae in the posterior midgut than the anterior midgut. Average passing time of gut content varies, in cockroaches (*Periplaneta*) it is 20 hours and in studies with grasshoppers it has been set to only a few hours (Chapman 2013).

Innervation of the gut

The stomatogastric nervous system innervate the muscles of the foregut and anterior midgut (Kirby & Clarke 1984; Chapman 2013). The stomatogastric nervous system varies widely between orders of insects. It is, however, fairly consistent within the orders and it is therefore likely that the morphology of the system in *A. domesticus* can be applicable to other orthopteran species (Kirby & Clarke 1984). The principal ganglion of this system is the frontal ganglion, located on the dorsal wall of the pharynx anterior to the brain. The principal ganglion connects to bilateral nerves called the frontal connectives. In orthoptera a median nerve, the recurrent nerve, extends beneath the brain to join the hypocerebral ganglion from which nerves extend to the ingluvial ganglions. There are two ingluvial ganglions, one placed laterally on each side of the crop. From the ingluvial ganglions nerves extend to innervate the midgut. In each ganglion there are central pattern generator circuits who coordinate the pattern of muscular contraction (Chapman 2013). The hindgut is anteriorly innervated by nerves extending from the stomatogastric ganglia and posteriorly innervated by the terminal abdominal ganglion (Kirby & Clarke 1984).

The alimentary canal of the house cricket, *A. domesticus*.

The alimentary canal of the house cricket is constituted by a large foregut, a small midgut and a large hindgut. In house crickets (five days old females), the foregut represents about 38% of the entire gut. The ventriculus of *A. domesticus* is small in size and plays a lesser role in digestion than in other insects. The ventriculus makes up 21% of the gut length and the hindgut makes up 41% of the gut length (Teo & Woodring 1985).

Previously described anatomical and histological features applies to the house cricket. Additional features are presented below.

Foregut; pharynx, esophagus, crop and proventriculus.

The transition from pharynx to esophagus is relatively clearly marked in the house cricket. There is a sharp reduction in the thickness of the surrounding muscle layer and the smooth and continuous intima gradually becomes covered with cones armed with spines. Posteriorly of this transition, extending into the neck, is a thin-walled dorsal evagination. The evagination appears transparent, the epithelial cells are small and appears to be buried in the intima. The muscle layer is thin and rarely exceeds one fiber in thickness. Rhythmic movements of the evagination moves fluid in and out of the main gut lumen and are independent to the rest of the esophagus. It is suggested that the main function of the evagination is to circulate the luminal content of the crop when being filled and showing little muscular activity. The dorsal parts of the esophagus are thinner than the ventral and have a less heavily armored intima. The dorsal and ventral parts are separated by a longitudinal invagination including intima, epithelium and longitudinal muscle layers. The fibers of the intrinsic circular muscle layer are separated from the gut wall and cover the external opening of the invagination concealing it from external view. The invagination runs along the entire length of esophagus, becoming shallower and finally emerging to the anterior part of the crop. When the esophagus is distended the invagination is straightened and the outer circular muscle layer is in contact with the gut wall (Kirby et al. 1982).

The gizzard is well developed and outlined with chitinous plates and teeth (Chapman 2013). Salivary glands of the house cricket are located on the floor of the haemocoel of the thorax and are composed of many lobes. They empty via a common salivary duct into the salivarium of the preoral cavity (Fox 2007).

Midgut; ventriculus with adjacent caeca.

There are two types of peritrophic membranes, type I, which is present in crickets, is produced along the entire midgut whereas type II is only produced by epithelial cells of the anterior midgut (Chapman 2013; Klowden 2013).

The house cricket has two caeca. The caeca are positioned laterally to each side of the gizzard, being equal in size of the gizzard when fully distended. Strands of connective tissue and muscle fibers connect the paired caeca to the gizzard and the narrow tube joining the gizzard to the crop. From the tip of each caeca a single strand extends to join the crop continuing to the connective tissue adjacent to the salivary glands and finally attaching to the exoskeleton of the neck region. The two strands are not running symmetrically, the left one joins the crop in two locations, and the right one only has one connection. The muscles of these strands are innervated by both the stomatogastric nervous system and the central nervous system (via the first thoracic ganglion) (Kirby et al. 1982).

Hindgut; pylorus, ileum, colon, rectum, anus.

The midgut of the house cricket contains bacteria (endosymbionts). The bacteria are associated to the peritrophic membrane and chitinous structures (Ulrich et al., 1981). The malpighian system of the house cricket is unusual. Since the ileum is hosting endosymbionts it would be unfavorable for the tubules to join the gut anteriorly of ileum. The gut content would be diluted, and the habitat would not be suitable for the hosted bacteria. In the house cricket the malpighian tubules join the gut in the posterior part of ileum (Hazelton et al. 1988).

Rotation of parts of the gut from an original, ancestral, position

Grylloidea and the Tettigonoidea are the only superfamilies within orthopteroid insects that have two caeca. The topographic placement of these differ between the infraorders. Relative to the gizzard, the caeca are located bilaterally in the Grylloidea, whereas they are located dorsally and ventrally in the Tettigonoidea. Studying the tracheation and innervation of the gut suggests that it has undergone evolutionary reposition. Abdominal spiracles from the left-handed side are joining the right-handed side of the esophagus and crop and the dorsal parts of the gizzard and caeca. Spiracles from the right-handed side are joining the corresponding side of each organ. The nerves and ganglia of *A. domesticus* demonstrate the same alteration, the ingluvial ganglions are being placed dorsally and ventrally rather than laterally. These observations suggest the caeca and gizzard being rotated 90° and the crop 180°, possibly an adaptation to the shape and proportions of the cricket body (Kirby et al., 1982).

Digestion

Digestion serves to provide the insect with essential energy and nutrients and refers to the chemical process where large and complex food molecules are degraded to smaller molecules that can be absorbed across the gut wall (Chapman, 2013).

Extra oral digestion

For many insects, the food can be partly or even fully digested before entering the oral cavity and the alimentary canal. Digestive enzymes are secreted, regurgitated on or injected into food and start to digest the food extra-orally. Enzymes used are deriving from the salivary glands or the midgut and include proteases and amylases. Using this technique enables conversion of solid food into liquid (Chapman 2013). In addition to digestive enzymes the saliva may contain anticoagulant and other pharmacological substances (Klowden 2013).

Digestion in the alimentary canal

Digestion is mainly mediated by enzymes, which have evolved alongside the insect and are well adapted to the specific diet of the insect. Carbohydrate digestion is mediated by carbohydrases, lipid digestion is mediated by lipases and protein digestion is mediated by endopeptidases and exopeptidases. Some food fragments, plant cell wall material, is in need of microbial fermentation to be broken down adequately (Chapman 2013). The anterior part of the hindgut may therefore work as a fermentation chamber (Klowden 2013). In some insects ileum host bacteria sited on elongated chitin spines (Chapman 2013; Klowden 2013). It has been shown that presence of bacteria increases digestibility of carbohydrates and contribute to increased growth rate (Kaufman et al., 1991). It is believed that the microbial flora is providing the host with digestive enzymes and vitamins and that may detoxify otherwise toxic plants (Klowden 2013).

Depending on their mode of action the digestive enzymes are concentrated to either the endo- or ectoperitrophic space. Initial digestion of macromolecules mediated by proteases and α -amylase occur in the endoperitrophic space whereas enzymes mediating the consecutive and final stages (acetylglucosaminidase, maltase, dipeptidase) take place in the ectoperitrophic space (Chapman 2013).

The crop is nearly impermeable to water and only sparse digestive activity take place here (Chapman 2013; Klowden 2013). The midgut is the main site of enzyme activity. Some insects, including the orthoptera, have substantial enzyme mediated digestion in the foregut (Chapman 2013). Foregut enzymatic activity is most likely mediated by ingested salivary enzymes and counter current movement of enzymes from the midgut (Teo & Woodring 1985; Cooper & Vulcano 1997; Chapman 2013). The caecae are serving to create a countercurrent flow of water in the midgut, optimizing digestibility (Klowden 2013). The activity of digestive enzymes are mainly influenced by temperature, pH and redox potential (Chapman 2013).

Temperature

Enzymatic activity generally increases with temperature. In most insect digestive enzymes the maximum activity range, *in vitro*, is between 35-45°C. These values also apply to species unable to cope with such high temperatures. In other words, the thermal limit of these species is not determined by the properties of their digestive enzymes (Chapman 2013).

pH

In most insects digestive enzyme optimum is at pH 6-7. The midgut pH is usually close to neutral, exceptions are the midgut region of the cyclorhaphous Diptera with pH 3-4 (lyse of dietary bacteria) and the opposite extreme midgut pH of the Lepidoptera larvae with pH exceeding 8 (uptake of H^+ by the midgut goblet cells). Due to secretions of the malpighian tubules the hindgut is slightly more acidic than the midgut (Chapman 2013). In insects with endosymbionts the pH is alkaline to provide a suitable environment for the bacteria (Klowden 2013). In the midgut of orthoptera the pH is ranging from 5.8-7.3 (Chapman 2013).

The pH value of the gut content in crickets vary depending on region and specie. In fed individuals of the black field cricket, *teleogryllus commodus* walker, the foregut is acidic, midgut neutral to slightly alkaline and the hindgut is alkaline. Type of food does not seem to affect pH. The crop content is acidic (possibly due to acidic salivary secretion) in fed individuals and neutral in starved. The crop content of starved individuals is possibly neutralized due to anterior fluid movement. Overall the pH increased in starved individuals. It is discussed whether it's the presence of food or some regulation that changes pH (Cooper & Vulcano 1997). In the house cricket the foregut content is acidic and the remaining content of the gut is close to neutral. In contrast to the black field cricket, crop content of starved individuals had a lower pH value compared to fed. The caecae secretes an alkaline fluid contributing to the increasing pH of the midgut and hindgut (Teo & Woodring 1985).

Redox potential

“Redox potential is a measure of the oxidizing or reducing condition in a system“(Chapman 2013). Most insects are believed to have a positive redox potential, taking into account the small size of the alimentary canal and the anaerobic conditions (Chapman 2013).

Digestive enzymes of *A. domesticus*

The luminal content of the crop and the ceecal tissue have a full set of enzymes. This suggests that the crop is a site of digestion, mediated by regurgitation from the caeca (Teo & Woodring 1985).

Carbohydrases

By hydrolysis of various carbohydrates by enzyme extracts prepared from different parts of the digestive tract of the house cricket Teo & Woodring (1985) could conclude that caeca is the main site for carbohydrase secretion. The caecal and foregut content show high levels of carbohydrase activity. Remaining sites of the alimentary canal hardly produces any carbohydrases (Teo & Woodring 1985).

Amylolytic activity was detected in all parts of the digestive tract except for the hindgut (Teo & Woodring 1985). The optimal pH for amylase was in the range of 5.6-6.4, suggesting that the crop with pH 5.5 ± 0.4 is the main site of amylase activity. Despite this Teo & Woodring (1985) found the amylase activity to be highest in the caecal tissue. Thomas and Nation (1984) made the same observation in the field cricket, *Gryllus rubens*. The luminal content of the anterior hindgut showed the highest amylase activity compared to content of other regions of the gut. In crickets starved for five days the concentration of amylase decreased with more than 44% (Teo & Woodring 1985). The amylase of the field cricket has a pH optimum around 7.5 (Thomas & Nation 1984) and is therefore more adapted to the slightly alkaline conditions of the midgut and hindgut than the amylase of the house cricket. The activity of the house cricket's amylase was shown to be highest at 40-45°C (Teo & Woodring 1985).

In 1992 Teo & Woodring showed that the optimal pH for maltase of the house cricket is 5.6 and it is only produced by the caecal tissue. The highest maltase activity was found in the foregut. House crickets being starved for five days resulted in a marked decrease of maltase activity. The maltase activity is increasing up to 45°C (Teo & Woodring 1992).

Raffinose hydrolyzing enzyme was the only carbohydrase to be shown present in the tissue of the anterior hindgut. Lactase could not be demonstrated in the gut of the house cricket. Not surprisingly since the normal cricket diet does normally not contain lactose. The house cricket usually feeds on plant materials containing large amount of cellulose. Despite this both cellulase and β -glucosidase was absent (Teo & Woodring 1985). Nevertheless the cricket can digest cellulose. The crickets have intrinsic cellulose activity deriving from ingested enzymes and the endosymbionts (Chapman 2013).

Lipase and protease

Performing hydrolysis of protein and lipid as net increase in absorbance by enzyme extracts prepared from different parts of the gut of house cricket Teo & Woodring (1985) showed that lipase was produced in all regions of the gut, except for the posterior hindgut. Lipase is the only enzyme produced by the anterior hindgut. The caecal tissue is the most active site for lipase and protease secretion. Protease was only found to be produced in the caecal and ventricular tissue (Teo & Woodring 1985). Using enzyme from complete digestive tract (content and tissue) the optimal pH for lipase and protease were determined to 7.6 and 8 respectively. In crickets starved for five days a marked decrease in lipase and protease activity was demonstrated (Teo & Woodring 1988). In the field cricket, both protease and amylase were detected in the tissue of the anterior hindgut (Thomas & Nation 1984).

Carbonic anhydrase, (CA)

CA is an enzyme family present in several tissues and organs in humans and a wide variety of animals. It contributes to many physiological processes for instance respiration, acid-base balance, bone resorption and calcification (Chegwidden & Carter 2000). The enzyme family was first discovered by Stadie & O'Brian (1933) and Meldrum & Roughton (1933) and serves to catalyze the reversible reaction of $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$. Today 16 different isoforms of CA have been described in humans (Frost and McKenna, 2014). CA have also been detected in birds (Holm et al. 2006), insects (Edwards & Patton 1967; Cooper & Vulcano 1997; Marc J. Klowden 2013) and spiders (Stratakis & Linzen 1984; Andersson et al., 2014).

In lepidopteran larvae a CA associated with the goblet cells produces carbonic acid, dissociating into a proton and a bicarbonate ion that give rise to high gut pH (Klowden 2013). In the black field cricket Cooper & Vulcano (1997) suggests that the acidic salivary secretion and alkaline midgut content is a result of CA activity. By electrometric methods on house cricket samples Edwards & Patton (1967) found CA in the tissues of the foregut, midgut, testes, salivary glands and fat body and possibly in the foregut contents, thoracic muscles and hindgut tissue. Starvation for 1 or 4 days did not seem to have any effect on the CA content of the foregut and midgut. Even though presence of CA is demonstrated in the alimentary canal of the house cricket its specific localization and function is still unknown and it remains unclear whether it contributes to acid or alkaline conditions (Edwards & Patton 1967).

MATERIAL AND METHODS

Animals

Crickets, *A. domesticus*, bred for zoo animal feed, were obtained from Herpers choice (Uppsala, Sweden) at 30 -35 days of age and held in a laboratory environment at room temperature with natural light cycle. The specimens were kept in transparent plastic boxes (39 x 28 x 28 cm), approximately 20 insects/ box, enriched with egg carton pieces for shelter. Cotton plugged glass tubes filled with water were put into the boxes in order to meet the water needs of the insects. They were fed ad libitum on conventional chicken feed, fresh fruits and vegetables.

Anatomy and histology

A preliminary study was made using ten individuals (five males and five females) at the age around 35 days. One male and one female were photographed for illustrations. Each one of the other eight individual was anesthetized with CO₂, decapitated, pinned to a cork disc with abdomen facing upwards and cut open longitudinally. Two individuals (one male and one female) were dissected under a binocular dissecting microscope. The gastrointestinal tract was dissected free from fat and photographed in situ using a Canon 7d mk II attached to the dissecting microscope. The entire tract was then removed, placed on a glass slide and photographed using the same camera. Three males and three females were then dissected and the gastrointestinal specimens were rapidly removed and fixed for 24h in buffered glutaraldehyde (2,5% glutaraldehyde in 1/15 M phosphate buffer, pH 7,2 at 4°C). The tissues were then rinsed with phosphate buffer. Dehydration was made by using graded ethanol (50%, 70%, 90%, 100%), 30 minutes in each solution. The samples were infiltrated with resin and 100% ethanol (1:1) for 3h and then put in resin for 24h. Specimen were embedded in Leica Histoiresin (Heidelberg, Germany), polymerizing at room temperature. Embedded specimens were serially sectioned in 2µm thickness using glass knives and a microtome (Leica RM 2165, Leica Instruments, Germany) and placed on glass slides. Sections from the entire gut from each specimen were stained with Haematoxylin Eosin (HE), mounted with agar100. These sections were evaluated using light microscopy to aid in the design of the main experiment and further dissection of cricket digestive tract.

The main study was performed when the insects were 40-45 days of age, when they were about to begin or finish their final molt. In total 20 insects were dissected, 10 fed and 10 starved (for 36h), equal distribution in terms of sex. All insects were euthanized and mounted for dissection as previously described. The gastrointestinal tract was rapidly removed, measured with digital calipers and weighted using Lab balance (Ohaus Scout Pro, Vetek Weighing AB, Vaddö, Sweden) before divided into one anterior (esophagus-proventriculus) and one posterior (caecum-rectum) fragment. For each individual, sex (male/female), treatment (fed/starved), wing status (no wings/ small wings/ fully developed wings), body weight, gut weight, body length (see Fig 2B), gut length, crop length, crop width, proventricular length and caecal length were recorded.

Collected data was processed; mean values, standard deviation, SEM were calculated. Students t-test (95 % of confidence) was performed to see if there were any significant differences between measurement from the fed and starved group or between sex.

All tissue fragments were fixed, embedded, sectioned, stained and mounted as described for the preliminary study.

Localization of CA activity

Gastrointestinal tracts fragments from two males and two females (age of 40-45 days) from the fed and starved group respectively, were sectioned and incubated for CA activity following Ridderstråle's method (Ridderstråle, 1991). Briefly the sections were incubated for 6 min floating on freshly prepared incubation medium containing 3.5 mM CoSO₄, 53 mM H₂SO₄, 11,7 mM KH₂PO₄ and 157 mM NaHCO₃. After incubation the sections were rinsed on 0.67 mM phosphate buffer (pH 5.9), transferred to blackening solution (0.5% (NH₄)₂S), and finally rinsed on two successive baths of distilled water. The incubation procedure results in a black precipitate of cobalt sulfide at sites of active CA. Before mounting some slides were counterstained with azure blue. The specificity of the reaction was checked with the CA inhibitor acetazolamide. Sections were first preincubated on a 10 µM solution of acetazolamide for 30 minutes and then incubated as previously described but with an incubating medium containing 10 µM inhibitor. All sections were photographed using a Nikon Microphot-FXA imaging system, (Bergström Instruments AB, Stockholm, Sweden).

RESULTS

Animals

Length of crickets used ranged between 14.4- 19.5 mm. Weight of the crickets used ranged between 0.18- 0.34 g. The wing status of the crickets used ranged from no wings to fully developed. The majority (13/20) had small, undeveloped wings.

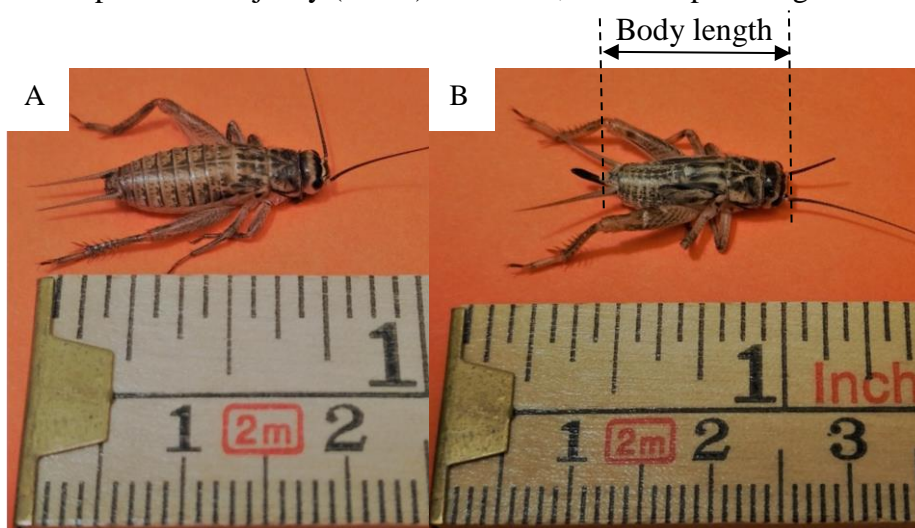


Fig. 2. A: House cricket, *A. domesticus*, male nymph. Age 30-35 days, two molts left before adult. B: House cricket, *A. domesticus*, female nymph. Age 30-35 days, two molts left before adult. Notice the ovipositor protruding from the posterior end of the abdomen.

Anatomy and histology

The alimentary canal of *A. domesticus* is composed of a large foregut, a relatively short midgut, with two adjacent caeca, and a large hindgut (see Fig. 3). The straightened alimentary canal is longer than the cricket itself ranging from 16.8- 28.0 mm. The crop is very variable in size, ranging from 2.9- 6.6 mm in length and 1.3- 2.6 mm in width. The rest of the components of the gut are fairly consistent in size, see Table 1.

Table 1. *Weight and measurements of fed and starved individuals*

	Male		Female	
	Fed(n=5)	Starved(n=5)	Fed(n=5)	Starved(n=5)
Body weight (g)	0.23± 0.05	0.25± 0.06	0.30± 0.06	0.27± 0.07
GI weight (g)*	0.04± 0.01	0.03± 0.01	0.06± 0.03	0.04± 0.02
Body length (mm)	16.2± 1.4	16.7± 1.2	17.3± 1.7	16.6± 1.4
GI length (mm)*	21.3± 4.4	21.7± 2.1	22.3± 5.1	21.1± 3.2
Crop length (mm)	3.9± 1.3	3.2± 0.3	4.6± 1.5	3.2± 0.2
Crop width (mm)	2.1± 0.4	1.7± 0.3	2.2± 0.6	1.9± 0.5
Proventriculus length (mm)	2.2± 0.2	2.3± 0.3	2.6± 0.4	2.5± 0.3
Caecum length (mm)	3.0± 0.7	2.7± 0.5	3.3± 0.8	3.1± 0.5

Data expressed as mean ± standard deviation (SD). T-test with 95% confidence interval was performed on mean values to compare the fed and starved group and males and females respectively. No significant results were given at $p < 0.05$, $n=8$. However, there was a tendency ($p=0.0693$) that the crop was longer in fed compared to starved females and that the proventriculus in fed males was longer compared to fed females ($p= 0.0569$). *Since the crickets were euthanized by decapitation the pharynx anterior part of esophagus is excluded.

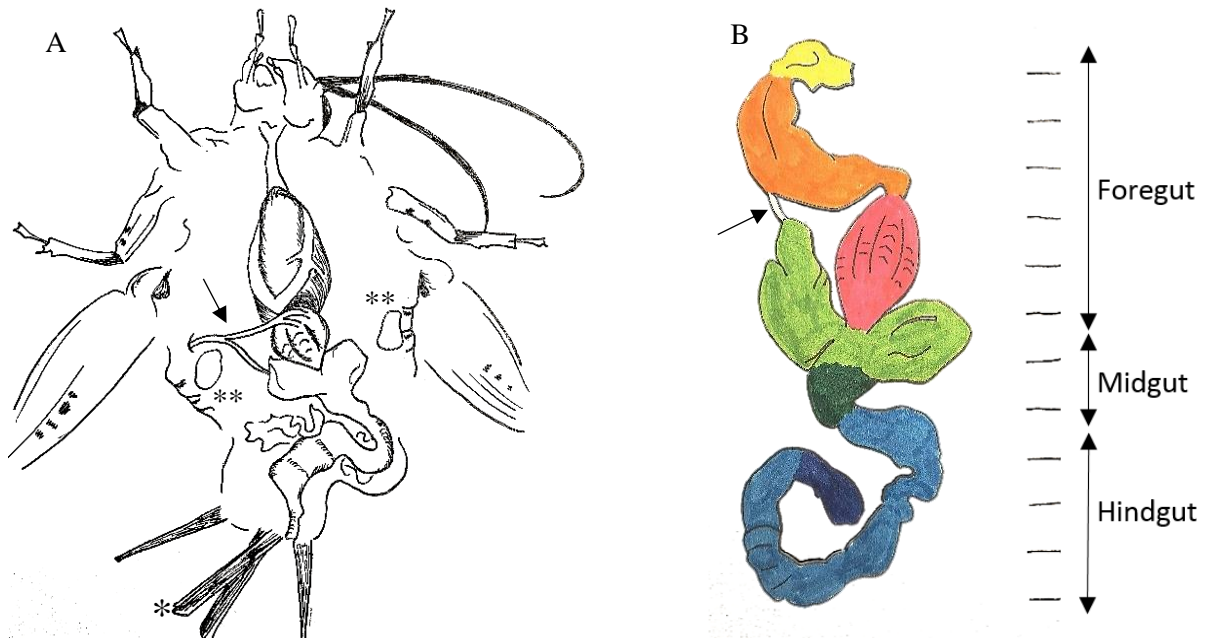


Fig. 3. The alimentary canal of the house cricket (illustrations by author). A: The alimentary canal in situ in a female house cricket, *A. domesticus*. Ovipositor* and ovaries ** visible. Note the spiracles (arrow) emerging from the right- handed side joining the ventral parts of the proventriculus. B: The alimentary canal of the house cricket, *A. domesticus*. Yellow: esophagus, orange: crop, pink: proventriculus, light green: caeca, dark green: ventriculus, light blue: ileum and colon, dark blue: rectum. Note the strand of connective tissue reaching from the tip of the caeca to the crop (arrow).

The foregut

The esophagus is formed by a simple epithelial layer covered with intima. Each epithelial cell is associated to a cone or spine (chitin teeth) pointing towards the posterior end of the gut. The epithelial cells are flattened, and the nucleus appears to be elongated. The epithelium is outlined by a thin muscle layer, approximately two fibers in thickness becoming thicker towards the crop. The esophagus exhibits several folds, including one longitudinal fold along the organs entire length (see Fig. 5).

The crop consists of a simple cuboidal epithelium, surrounded by muscle fibers, forming both small and large folds. The small folds of the epithelium give rise to elevated pads protruding into the lumen. Each epithelial cell is associated to a cone or spine (chitin teeth) pointing towards the posterior end of the gut. The surrounding muscle layer is approximately five fibers in thickness (see Fig. 6).

The proventricular epithelium is simple cuboidal or slightly cylindrical, and is outlined by intima. The intima differs from the one presented in esophagus and the crop. Instead of cones and spines there is a smooth chitin layer with thin hair-like bristles associated with the epithelial cells. The proventricular epithelium is surrounded by a very thick muscle layer, approximately 15 fibers in thickness (see Fig. 7). The proventriculus exhibits six marked longitudinal ridges (see Fig 4). Each ridge possess symmetrical folding. The ridges are constituted by the proventricular epithelium outlined with intima and have a muscular core. The ridges are separated by stiff longitudinal chitinous spines. Bilaterally of the proventriculus the two caeca are located (see Fig. 2).



Fig. 4. *Proventriculus of house cricket cut open longitudinally, inside facing. Longitudinal ridges (arrow), six in total, formed by the epithelium.*

The midgut

The midgut is more complex than the foregut. Excessive folds of the epithelium and very sparsely of surrounding muscle fibers are the main characteristics of the caecal tissue. The epithelium is formed by simple columnar cells. Regenerative stem cells are assembled in nidi, close to the basal lamina. Correlated to the placement of the nidi there are areas, adjacent to the lumen, with oval cells arranged in groups. These cells stain very lightly with HE, appear to contain droplets, and are likely goblet cells. The groups of goblet-like cells may be perceived as gastric glands. The caecal lumen is packed with granules (see Fig. 8). The ventriculus resembles caecum but is not as heavily folded. In the junction between the midgut and the hindgut the epithelium starts forming club like protrusions with chitinous spines radiating from it (see Fig 9).

The hindgut

In ileum the epithelium is cuboidal, outlined with intima and surrounded by a very thin muscle layer, a few fibers in thickness. Ileum is hosting endosymbionts, bacteria associated to the peritrophic membrane and club-like epithelial protrusions. From each epithelial club long spines are originating, radially, stretching into the peritrophic membrane (see Fig. 10).

Gradually the spines of the club-like protrusions become less distinct and ultimately disappearing at the posterior end of ileum. Left is a folded epithelium lined with a smooth chitin layer. The surrounding muscle layer is approximately four fibers in thickness becoming thicker towards the rectum (see Fig 11). Rectum resembles the posterior part of ileum, except for the epithelium being simple cylindrical cells and the surrounding muscle layer being approximately twice as thick. Areas of the epithelium are presented with more elongated cells, forming elevated pads (see Fig. 12).

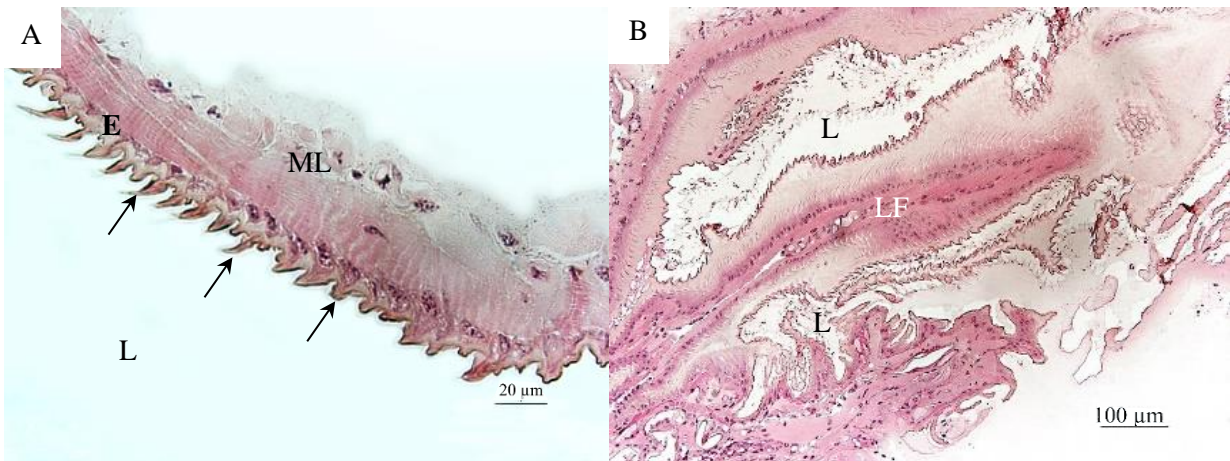


Fig. 5. *Esophagus from house cricket. A: Esophagus of female cricket. Epithelium and surrounding muscle layer with inner longitudinal muscle fibers and outer circular muscle fibers. Chitin teeth associated with the epithelial cells pointing towards posterior end of the gut (arrows). B: Esophagus of male cricket, longitudinal cut. Central longitudinal fold reaching long the entire length of the organ. Epithelium (E), Muscle layer (ML) Lumen (L), Longitudinal fold (LF)*

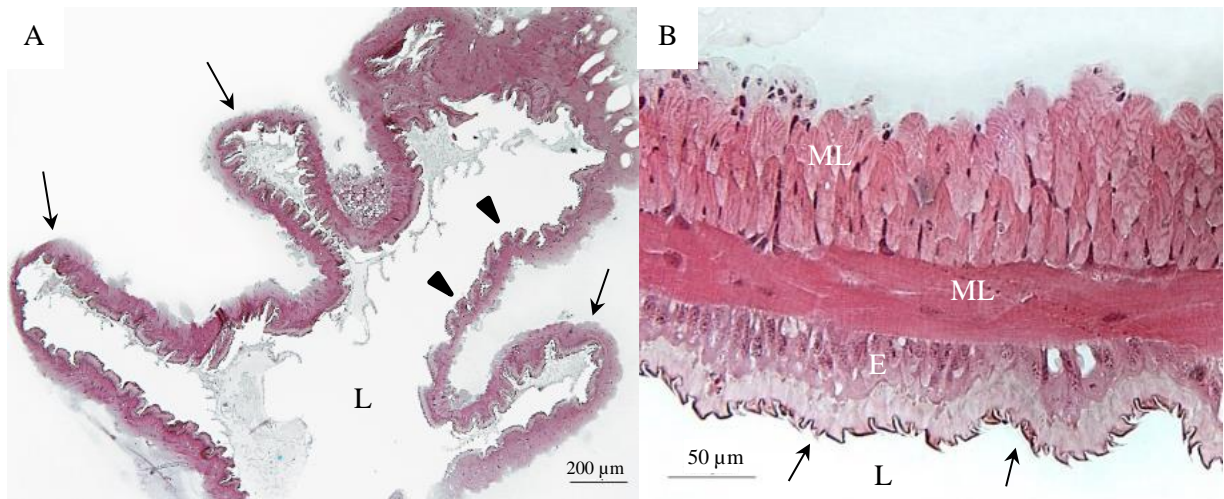


Fig. 6. *Crop from female house cricket. A: The entire organ presented with several large folds (arrows and numerous small folds (arrowheads)). B: Epithelium with surrounding muscle layer with inner longitudinal muscle fibers and outer circular muscle fibers. Chitin teeth associated with the epithelial cells pointing towards posterior end of the gut (arrows). Epithelium (E), muscle layer (ML), Lumen (L)*

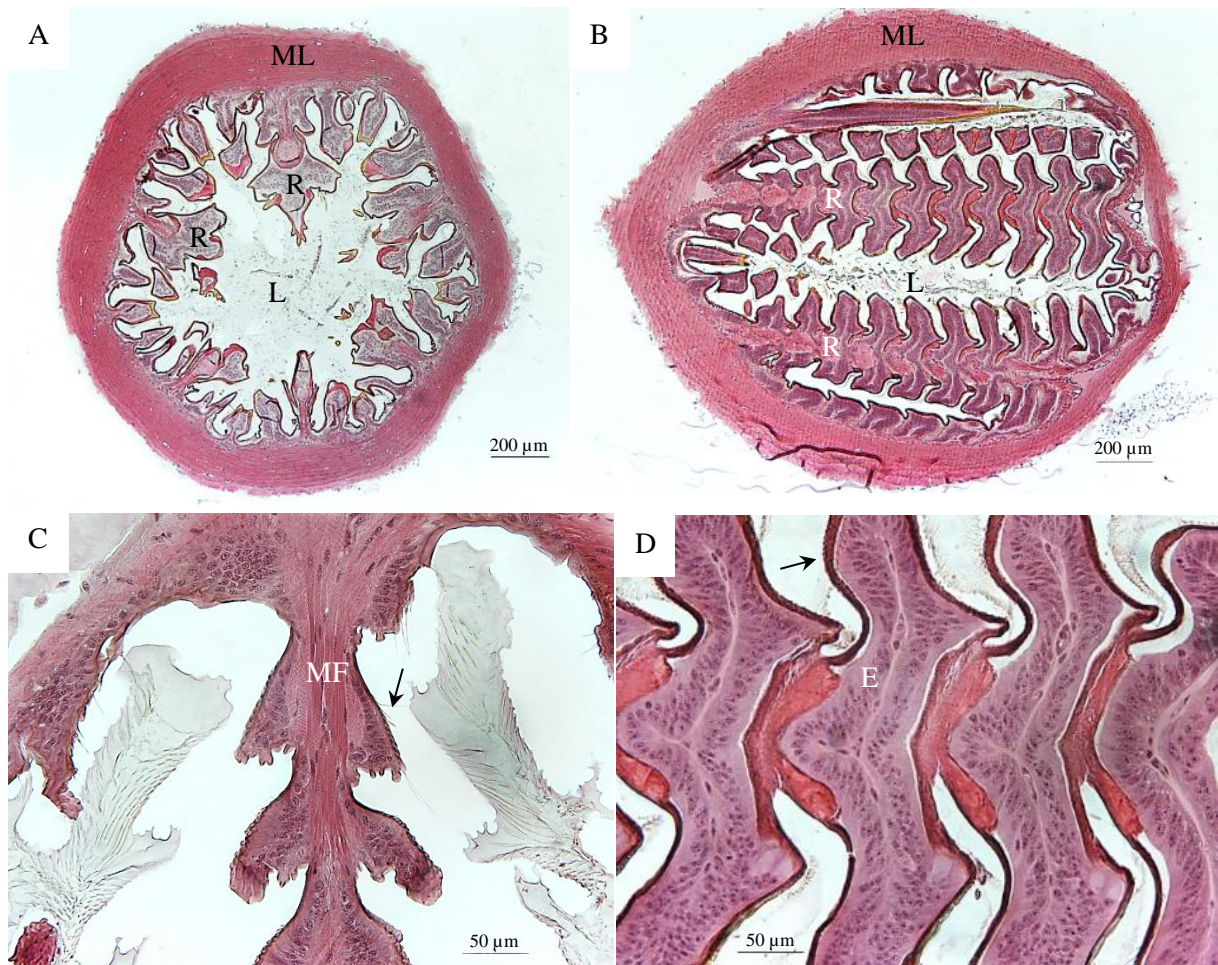


Fig. 7. Proventriculus from house cricket. A: Proventriculus of female cricket. The entire organ, transversal cut. Epithelium forming ridges and spines outlined by intima surrounded by a thick muscle layer. B: Proventriculus of female cricket. The entire organ, longitudinal cut. Longitudinal ridges stretching along the entire length of the organ. C: Proventriculus of male cricket. Single ridge, longitudinal cut. The ridge is constituted by symmetric folds with bristles (arrow) and has a muscular core. D: Proventriculus of female cricket. Ridge, longitudinal cut. Symmetric folds formed by epithelium outlined by intima (arrow).

Ridges (R), Spines (S), Muscle layer (ML), Lumen (L), Muscle fibers (MF)

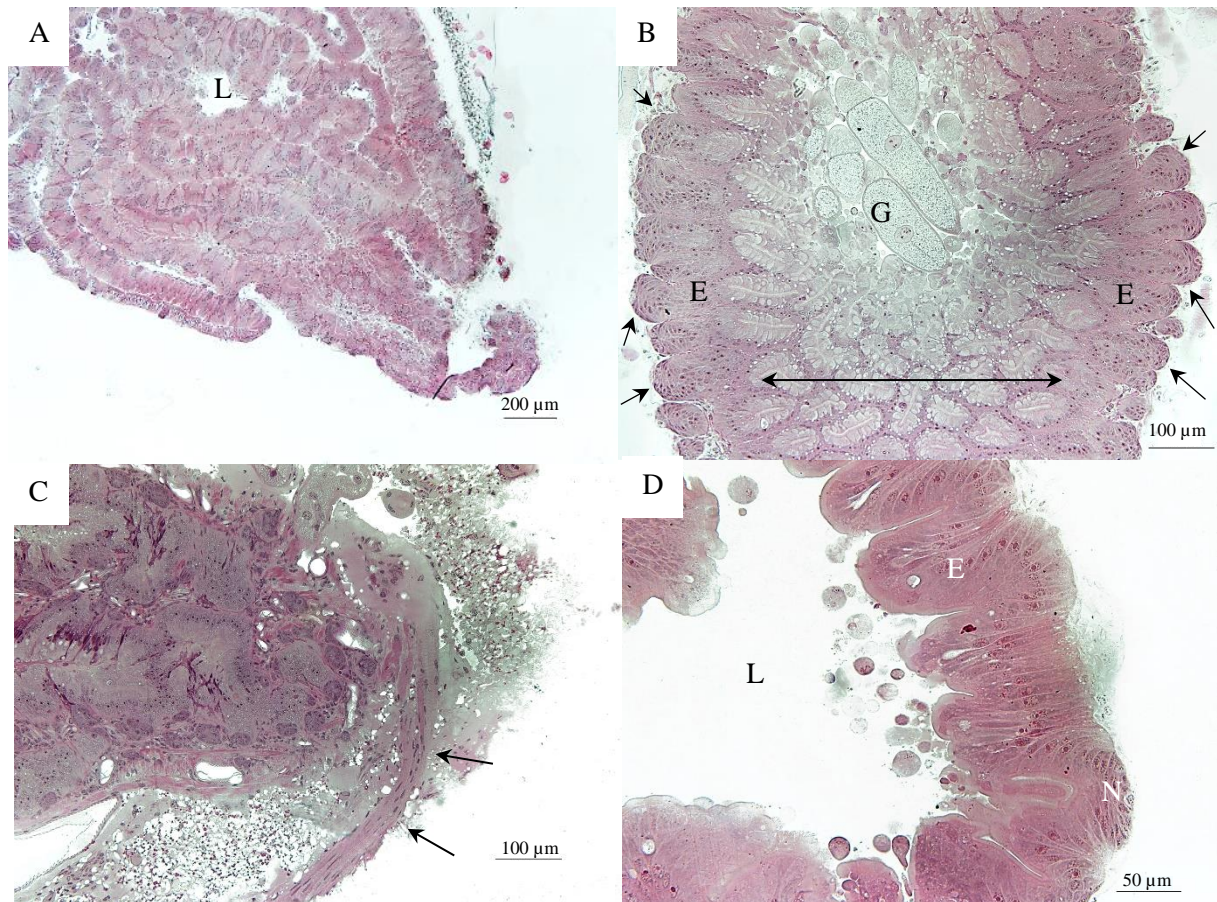


Fig. 8. Caecum from house cricket A: Caecum of male cricket, caecal tip. The organ exhibits excessive epithelial folds. B: Caecum of female cricket, transversal cut. Bilaterally in the photo you see the outer walls of the caeca. The caecal epithelium is heavily folded and presented with numerous nidi (arrows). Centrally goblet-like cells are arranged in a gland like appearance (area between double headed arrow). The lumen is packed with granules (G). C: Caecum of male cricket. Caecal tip with strain of connective tissue and muscle fibers which attaches the caeca to the proventriculus, crop and thorax (arrows). D: Caecum of male cricket. Caecal epithelium with nidi. Epithelium (E), Nidi (N), Lumen (L), Granules (G)

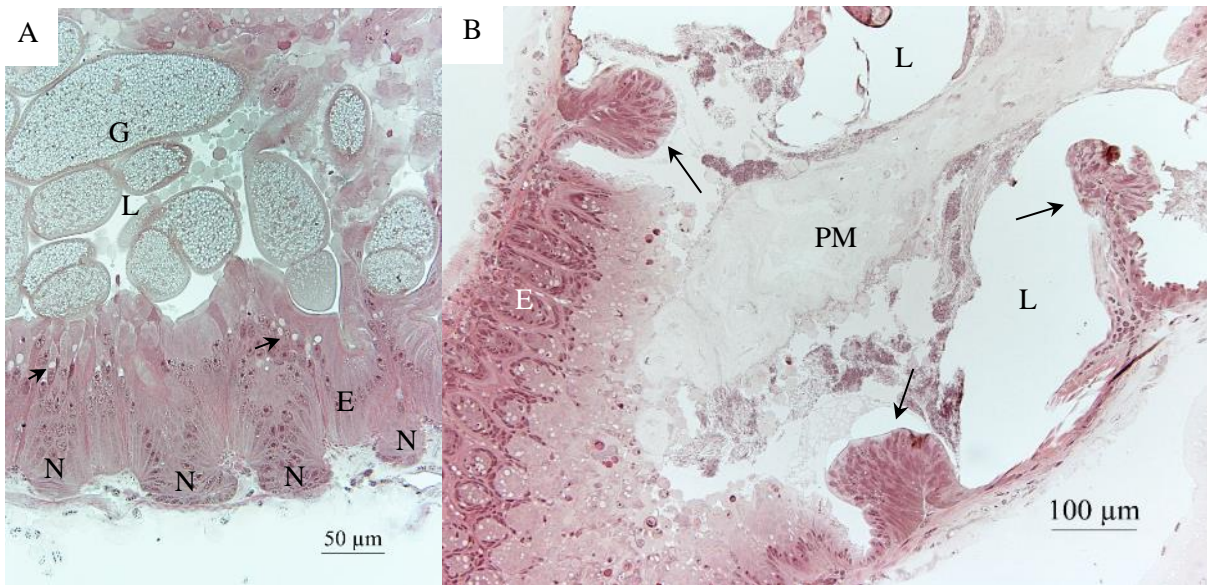


Fig. 9. Ventriculus from female house cricket A: Epithelium with columnar epithelial cells and more apically, adjacent to the lumen goblet-like cells (arrows). Lumen is packed with granules B: Junction of ventriculus- ileum. Transition from heavily folded epithelium to a less folded with club-like protrusions (arrows). The peritrophic membrane becomes thicker and more prominent towards the posterior parts of the gut. Epithelium (E), Nidi (N), Granules (G), Lumen (L), Peritrophic membrane (PM)

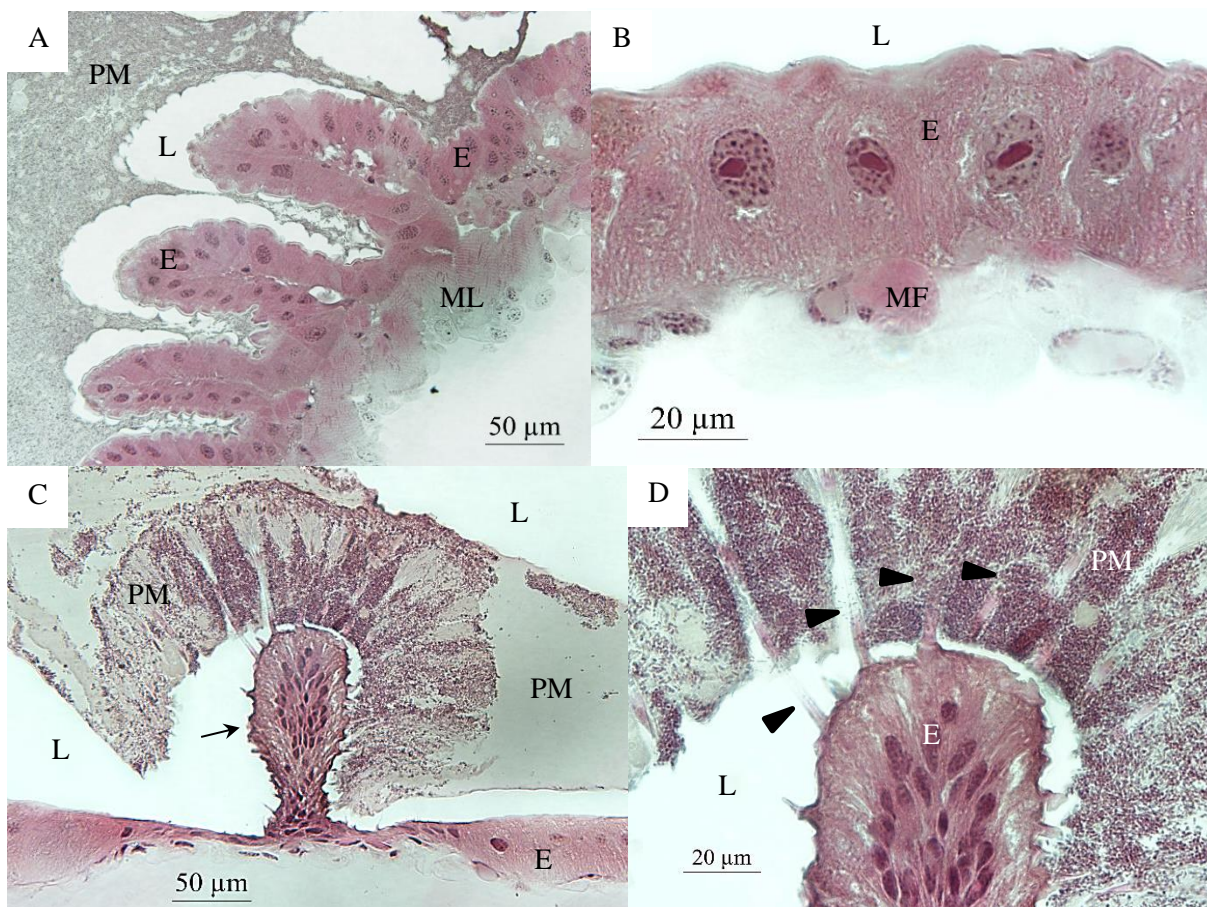


Fig. 10. Ileum from house cricket A: Ileum of male cricket. Epithelium with club-like protrusions and surrounding muscle layer. B: Ileum of female cricket. Cuboidal epithelium. C: Ileum of female cricket. Club-like protrusion (arrow) with associated endosymbionts (bacteria). The endosymbionts are found on spines radiating from the protrusion into the peritrophic membrane D: Ileum of female cricket. Tip of club-like protrusion with associated endosymbionts (bacteria). Spines (arrowheads) radiating into the peritrophic membrane. Epithelium (E), Muscle layer (ML), Lumen (L), Muscle fiber (MF), Peritrophic membrane (PM)

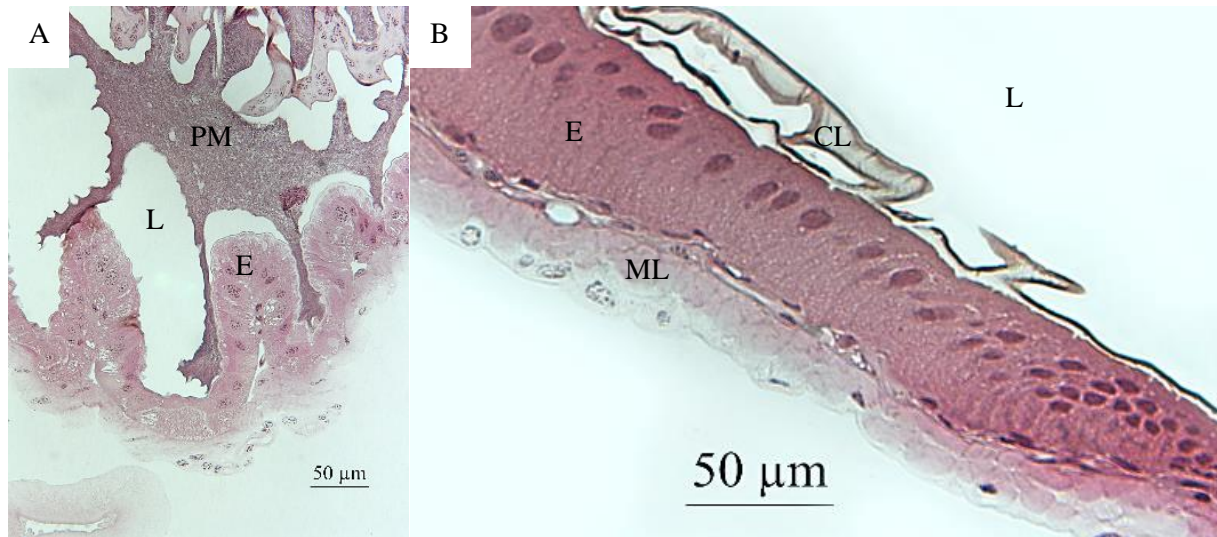


Fig. 1. *Posterior ileum from house cricket. A: Ileum of female cricket. Epithelium, the club-like protrusions gradually becomes less armored and the spines disappears. B: Ileum of male cricket. The epithelium becomes simple cylindrical and lined with a smooth chitin layer towards rectum. Epithelium (E), Muscle layer (ML), Lumen (L), Chitin layer (CL)*

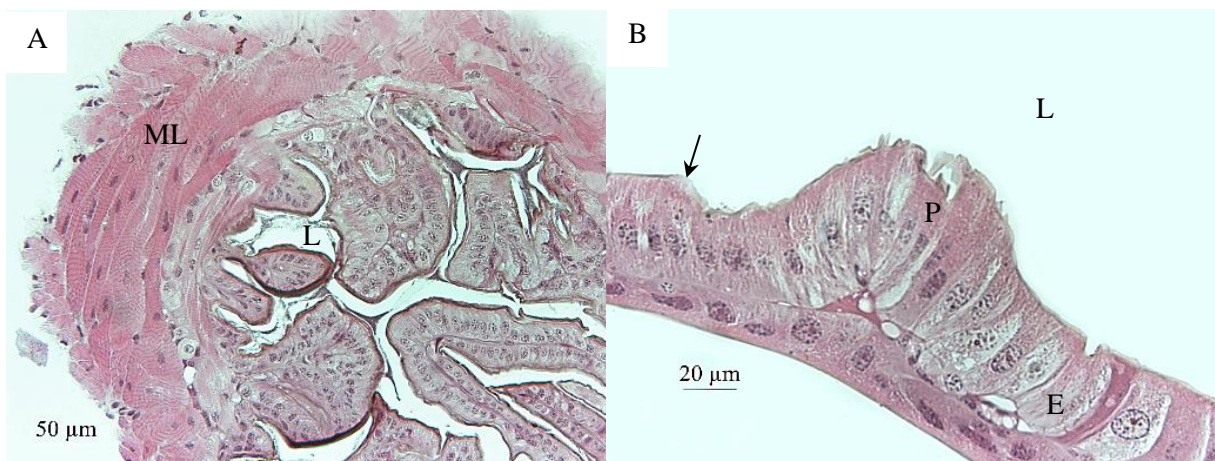


Fig. 12. *Rectum from house cricket A: Rectum of male cricket, transversal cut, folded epithelium with smooth chitin layer surrounded by a thick muscle layer. B: Rectum of female cricket. Simple cylindrical epithelium lined with a thin chitin layer (arrow). Groups of more elongated epithelial cells form rectal pads. Epithelium (E), Muscle layer (ML), Lumen (L), Rectal pad (P)*

Localization of carbonic anhydrase

Active CA is demonstrated by cobalt precipitation as a black staining. Sections incubated with the inhibitor were generally unstained, but on some section certain structures contained traces of black stain. These areas contained chitinous structures and bacteria and were judged not to contain CA.

Membrane-bound CA is found in the striated musculature surrounding the epithelium of the gut. CA is demonstrated in both longitudinal and circular muscle fibers in esophagus, crop, proventriculus, caeca, ventriculus, ileum, colon and rectum. The CA is present in the entire membrane radiating towards the center of the cell forming a spoke-like pattern (see Table 2A, Fig. 13).

In the caecal and ventricular tissue black stain is, in addition to the striated musculature, present in the epithelial layer (see table 2B). Black staining is found in the basolateral parts of the membrane in most of the columnar cells and is fading towards the cell's apical end. Throughout the epithelium there is a repetitive pattern of areas with no black staining which represent nidi and groups of immature cells. Each group of immature cells is bilaterally surrounded by mature columnar epithelial cells with black membrane staining. As seen in sections stained with HE (Fig. 9); directly above each nidi, adjacent to the lumen, there is a small group of cells that are oval and very lightly stained. These groups of cells resembles goblet cells forming glands and do not contain black staining. The CA activity is found in both fed and starved individuals with exception of ventricular and possibly caecal epithelium in starved males (see table 2, Fig. 13).

Table 2A. Carbonic anhydrase activity in muscle tissue in different parts of the gut of male and female house cricke, (- \rightarrow +++). - equals no activity and +++ equals marked activity. Blank, no data available.

	Male		Female	
	Fed	Starved	Fed	Starved
Esophagus	++	+++		++(+)
Crop	++	+++	++	+++
Proventriculus	++(+)	++(+)	+++	++(+)
Caecum	++(+)	++(+)	++(+)	+++
Ventriculus	++(+)	+++	+++	+++
Ileum	++	++(+)	++	+++
Colon	+(+)	+++	++	++
Rectum				+++

Table 2B Carbonic anhydrase activity in the epithelial layer in caecum and ventriculus of male and female house cricket (- \rightarrow +++). - equals no activity and +++ equals marked activity.

	Male		Female	
	Fed	Starved	Fed	Starved
Caecum	++(+)	(+)	++(+)	+++
Ventriculus	++(+)	-	+++	+++

Fig. 13. (see p. 22) Active carbonic anhydrase in the alimentary canal of house cricket seen as black staining (cobalt precipitation) with azure blue counterstain. **A:** Esophagus of starved female showing weak to moderate black membrane staining in muscle layer. **B:** Crop of starved male showing strong black membrane staining in muscle layer. **C:** Junction proventriculus-caecum of starved male with strong black membrane staining in muscle layer. Note that the proventricular muscle layer is thicker than the caecal muscle layer. No CA activity in caecal epithelium. **D:** Caecum of fed female with weak to moderate black membrane staining in muscle layer and epithelium. No CA activity is seen in groups of stem cells (nidi) and immature epithelial cells. Apical weak to moderate black membrane staining basolateral in epithelial cells. Goblet cells are present close to the lumen (arrows). **E:** Caecum of fed female with strong black membrane staining in muscle layer and weak to moderate black membrane staining basolateral in the epithelial cells. Repetitive pattern of areas with no black staining represent groups of stemcells/ immature cells. **F:** Ventriculus of fed female with weak to moderate black membrane staining in muscle layer and epithelium. No CA activity is seen in groups of stem cells and immature epithelial cells. Laterally and apically of each area with no black staining weak to moderate black membrane staining basolateral is seen in epithelial cells. **G:** Ileum of starved female with strong black membrane staining in muscle layer. **H:** Rectum of starved male with moderate to strong black membrane staining in muscle layer presented in a spoke-like pattern. Epithelium (E), Muscle layer (ML), Lumen (L), Peritrophic membrane (PM), Nidi (N), Immature cells (IC), Mature epithelial cells (MC)

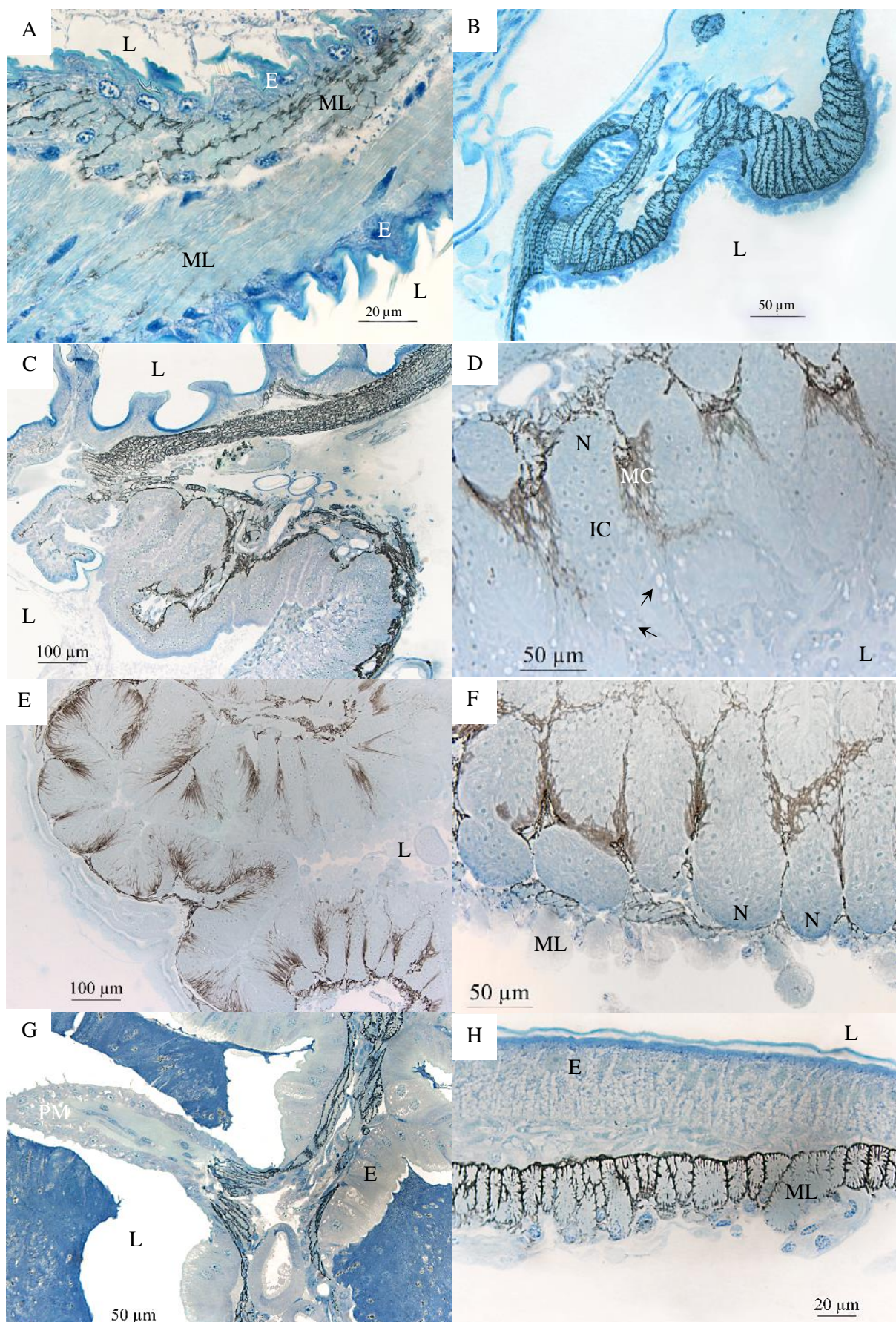


Fig. 13. Carbonic anhydrase in the alimentary canal of the house cricket, *A. domesticus*. See page 21 for description.

DISCUSSION

In the literature solely the midgut of *A. domesticus* has previously been histologically documented (Ulrich 1981). The gut of other species within the Gryllidae have been investigated, also mainly focusing on the midgut (Woodring & Lorenz 2007; Biagio 2009; Cakici 2012). In this study the anatomy and histology of the entire digestive tract has been described. The results show that the anatomy of the digestive tract of the house cricket very much resembles the digestive tract of other species within the Gryllidae family (Fernanda 2009, Woodring & Lorenz, 2007, Özlem 2012). Studying the histology of the house cricket's gut shows that the intima which lines both the esophageal and crop epithelium forms chitin teeth pointing towards the posterior end of the gut. It is believed that the direction of which the teeth are pointing is contributing to the forward flow of gut content (Chapman 2013). In 1982 Kirby for the first time described the anatomic structures of a dorsal evagination of the esophagus, an esophageal groove and caecal attachment and presented evidence of evolutionary gut rotation in *A. domesticus*. Three of these features; the esophageal groove, the caecal attachment and evidence of evolutionary rotation, have now been further documented in this study. The proventriculus of *A. domesticus* has six internal ridges (see Fig. 4), also seen in *G. bimaculatus*. In the ileum of *G. bimaculatus* Woodring (2007) describes fingerlike invaginations associated with endosymbionts. These invaginations can most likely be correlated to the club-like protrusions seen in the ileum of *A. domesticus* in this study. The club-like protrusions in *A. domesticus* have earlier been described by Ulrich et al., (1981). In HE stained sections the different cell types of the midgut epithelium cannot be fully differentiated. The columnar epithelium is most likely dominated by enterocytes. Nidi, groups of stem cells, are present throughout the epithelial layer close to the basal lamina. There are areas, adjacent to the lumen, with goblet-like cells arranged in groups. Areas of similar appearance are described as gastric glands in *Melanogryllus desertus* (Cakici 2012) suggesting that such glands may be present in *A. domesticus* as well. To confirm the presence of goblet cells and investigate the constitution of the possible gastric glands additional histological examination with PAS-staining is needed.

In this study the rectum is presented with areas of elongated cells forming rectal pads. These areas are thought to absorb water and ions (Chapman 2013; Klowden 2013). If the elongated cells of the rectal pads are of a different cell type than the cells of the rectal epithelium has not been investigated. It is suggested that the intima covering the rectal pads is thinner (Chapman 2013, Klowden 2013). In this study the intima appears to be consistent in thickness along the entire length of the rectal epithelium.

Weights and measurements from 20 individuals were used in the main study, five individuals in each group (fed males/ fed females/ starved males/ starved females). The different measurements collected were selected to obtain as consistent values as possible. During the study the general impression was that females were bigger than males regarding both body size and gut size. In the literature there are vague implications that this would be the case. Hobby web-pages claim that females are or are likely to be bigger than males (Naveen 2012; insect identification 2014) whereas a more reliable source claims that this is not the case (Strid et al., 2017). No significant results were obtained regarding differences between groups including males compared to females and fed individuals compared to starved. However, there was a tendency ($p=0.0693$) that the crop was longer in fed compared to starved females and that the proventriculus in fed males was longer compared to fed females ($p= 0.0569$). The crickets in the study were euthanized via decapitation, therefore pharynx and the most anterior parts of the esophagus were not included in the data. When stretching out the entire gut to measure the total length the gut immediately started to contract, to retake its original form, resulting in some inconsistency. The age of the crickets used differed with approximately ten days. The youngest had two molts left before becoming adults and the oldest had recently finished their last molt. It is likely that the small groups, some inconsistency during measurements, varying age and observational bias contributed to the results. If the study is repeated more care should be taken to have crickets of as similar age as possible, using a blind study design and increase the size of the groups, the latter which was not possible in this project for practical reasons.

In this study CA has been demonstrated to be present along the entire length of the alimentary canal of the house cricket. It is seen in the membrane of the muscle fibers outlining esophagus, crop, proventriculus, caeca, ventriculus, ileum and rectum presenting a spoke-like pattern in the cells. The pattern can possibly be explained with the configuration of muscle fibers. The spokes may represent T-tubules which are membrane invaginations serving to conduct electric impulses through the fiber. The presence of CA may increase the tissues ability to isolate CO_2 formed during exercise preventing harmful effects (Edwards & Patton 1967). In the midgut CA is, in addition to the musculature, present in the epithelial layer. Black staining is found in the basolateral parts of the membrane in most of the columnar cells. Throughout the epithelium there is a repetitive pattern of areas with no black staining which represent nidi and adjacent groups of immature cells. Each group of immature cells is bilaterally surrounded by mature columnar epithelial cells with black membrane-bound staining. The described staining pattern suggests that the stem cells wander towards the lumen and move laterally as they differentiate to epithelial cells. CA have been demonstrated in different cell types of the gut, including mammalian parietal cells (Davenportj 1939) and goblet cells of lepidopteran larvae (Klowden 2013). Since the CA appears to be membrane bound to the majority of the columnar epithelial cells it is likely that the CA activity is associated to the enterocytes (the most numerous cell type) of the midgut. In *A. domesticus* the stem cells, the immature epithelial cells and earlier described goblet-like cells do not contain black staining. In order to determine which cell type/ cell types that contains CA additional histological examination is needed.

Note that the method used to demonstrate CA gives cobalt phosphate precipitation from all isozymes, but is limited to the active form of the enzyme. Not yet activated or inactivated CA cannot be detected with the cobalt phosphate precipitation method (Ridderstråle 1991). Data is lacking regarding the esophagus in fed females and the rectum of both fed and starved males and fed females. Studying the HE stained samples of these fragments we know that there is presence of striated musculature. It is likely that the musculature exhibits CA activity, but we cannot conclude this until supplementary observations have been made.

By electrometric methods Edward and Patton (1967) measured the hydration of carbon dioxide following injection of gut tissue and gut content into CO₂ – saturated water. They demonstrated that CA was present in the foregut tissue, midgut tissue and content and possibly the foregut content and hindgut tissue and/or content of *A. domesticus*. In this study we have set the localization of active CA to the muscle layer of the foregut tissue, the epithelium and muscle layer of the midgut and the muscle layer of the hindgut. Studying digestive enzymes of the house cricket Teo & Woodring (1985) found that midgut secretions moved forward into the foregut. Injecting dye, Woodring (2007) showed anterior movement of ceecal content in starved or recently molted female individuals of *G. bimaculatus*. The same forward movement could not be demonstrated in fed individuals. Edwards and Patton's (1967) observations of possible CA activity in foregut content can be explained by the presence of CA in the midgut epithelium and the anterior movement of midgut content. The mechanism of which creates the counter flow is still unknown. Edwards & Patton (1967) did not see any changes in CA activity when crickets were starved for one or four days. In this study comparable levels of CA activity are found in both fed and starved individuals with exception of ventricular and possibly caecal epithelium in starved males. The males showed none to weak black staining compared to the much stronger staining in the epithelium of females, raising the question if there are sex differences. Note that the study was not blinded, the observer did know if the individuals were fed or not. In mammals CA activity appears to be affected by the concentrations of sexual hormones. Sex differences of CA III have been observed in the liver of rats. The concentration CA III is much higher in male rats compared to females and ovariectomized females receiving testosterone show an increased CA III concentration in the liver (Shiels et al., 1983). Cytosolic CA in the duodenal mucosa in rats is responsive to estradiol propionate and CA activity were increased by administration of the substance (Suzuki et al., 1991). These studies show that at least in rats CA activity may be altered by sex hormones. Interestingly one study show such effects in the digestive tract in rats (Suzuki et al., 1991). To confirm the results in *A. domesticus* a larger blinded experiment with more individuals is needed.

The foregut content of the house cricket is acidic whereas the remaining gut content is close to neutral. Foregut pH is decreasing when individuals being starved (Teo & Woodring 1985). It is unclear how the pH gradient along the gut is established. Most likely many factors contribute, including presence of food and acidic salivary secretions as suggested in *teleogryllus commodus* walker (Cooper & Vulcano 1997), alkaline midgut secretions as well as gut peristalsis (Teo & Woodring 1985). Since CA catalyzes the reversible reaction of $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$ one of its main contributions is acid-base balance (Chegwidden & Carter 2000). In this study we have shown presence of CA in midgut epithelium suggesting that CA may contribute to the alkaline secretions observed by Teo and Woodring (1985). To test this hypothesis pH can be determined in the midgut using ion selective microelectrodes as performed on the silk gland of spiders. By using CA inhibitors, and results from histochemical staining of CA as performed in this study, the researchers were able to conclude that CA was involved in the pH gradient created in the major silk gland (Andersson et al., 2014).

CONCLUSIONS

In this study the entire alimentary canal of *A. domesticus* has been described, both anatomically and histologically, for the first time. The alimentary canal of *A. domesticus* is very similar to the gut of other species within the Gryllidae family. The results suggest the presence of goblet cells forming gastric glands in the midgut epithelium. To confirm the presence of goblet cells and investigate the constitution of the possible gastric glands additional histological examination with PAS-staining is needed. During the study the general impression was that females were bigger than males regarding both body size and gut size. However, no significant results were obtained regarding differences between groups including males compared to females and fed individuals compared to starved. If the study is repeated more care should be taken to have crickets of as similar age as possible and increase the size of the groups.

The localization of active CA has been demonstrated in the striated muscle along the entire alimentary canal and in the epithelium of the ceaca and ventriculus. CA activity is found in the membrane of the majority of the columnar epithelial cells in the midgut suggesting that CA is associated to the most numerous cell type, the enterocyte. In order to determine which cell type/ cell types that contains CA additional histological examination is needed. There are possible sex differences regarding CA activity in the midgut. Starved males showed none to weak black staining compared to the much stronger staining in the epithelium of starved females. To confirm the results of possible sex differences the study needs to be repeated with increased group sizes. It is suggested that CA in the alimentary canal of *A. domesticus* may contribute to acid-base balance in both muscle and gut lumen. Future experiments with ion selective microelectrodes and presence/ absence of CA inhibitors may be a way to test this hypothesis.

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